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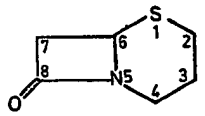
(54) IMPROVEMENTS IN OR RELATING TO CEPHALOSPORIN COMPOUNDS

(71) We, GLAXO LABORATORIES LIMITED, a British Company, of Greenford, Middlesex, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed to be particularly described in and by the following statement:—

This invention relates to a process for obtaining compounds which are useful in the preparation of antibiotics in the cephalosporin series. In particular the present invention relates to a process for N-deacylating cephalosporin compounds.

The compounds referred to in this specification are generally named with reference to cepham which has the structure



(see *J.A.C.S.* 1962, *84*, 3400).

The N-deacylation of 7β - acylamino cephalosporin compounds is an important step in the production of cephalosporin antibiotics. Various methods of acylating 7β-aminocephalosporin compounds are available and so a general method of N-deacylating 7β-acylamino cephalosporin compounds would allow one to introduce and remove acyl substituents at any desired stage in the synthesis of cephalosporin antibiotics.

Several general methods of N-deacylating cephalosporin compounds have been proposed including direct acid hydrolysis using e.g. 0.1N to N hydrochloric acid and a method of N-deacylating a 7β-acylaminocephalosporanic acid in an organic solvent with a reagent which acts as an external source of alkyl carbonium ions as is described in British Patent Specification No. 1,070,312. However, both these methods yield the 7β-amino compounds in low yields.

Methods of deacylating specific 7β-acylamino cephalosporin compounds have been proposed with a view to obtaining the corresponding 7β-amino compound in improved yields. Such methods include the N-deacylation of cephalosporin C and derivatives thereof using nitrosyl chloride such as is described in British Patent Specification No. 1,017,534 and the imide halide technique for N-deacylating such compounds as is described in Specification No. 1,041,985. The nitrosyl chloride technique is dependent upon the specific nature of the δ-aminoadipoyl acyl group in cephalosporin C.

Among the various methods available for N-deacylating cephalosporin compounds we have surprisingly found that the imide halide method is of broad application and thus enables one to obtain a good yield of the corresponding 7β-amino derivatives from a wide variety of 7β-acylamino cephalosporin compounds generally irrespective of the nature of the 7β-acylamino group. The process is particularly applicable to the N-deacylation of 7β-acylamino cephalosporin compounds obtained by synthetic or semi-synthetic routes. Thus the process is applicable to the N-deacylation of 7β-acylamino cephalosporin compounds obtained by transformation of 6β-acylamino penicillin analogues (see, for example, United States Patent Specification No. 3,275,626) such acyl group being, for example, phenoxyacetyl or phenylacetyl.

According to the present invention, therefore there is provided a process for the N-deacylation of a 7β-acylamino-4-carboxy-

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cephalosporin compound (other than one having at position-7 a δ-aminoadipoylamin group or a δ-aminoadipoylamino group having one or other or both of the amino and carboxy groups thereof blocked) which comprises converting into an imide halide by means of an imide halide forming reagent, converting the imide halide so formed into an imino ether and hydrolysing or alcoholysing the latter to produce a 7β-amino cephalosporin compound.

In carrying out the present process the 4-position carboxy group of the starting material should be blocked prior to conversion to the imide halide, for example as described hereinafter. Other functional groups which may be present may also be blocked as necessary.

Examples of 7β-acylamino cephalosporin starting materials which may be N-deacylated in accordance with the invention are compounds having a methyl group at the 3-position and may be prepared using the method described in British Patent Specification No. 1,121,308. The starting material may be a 3-acetoxymethyl compound in which event it may be derived from cephalosporin C. 3-Acyloxymethyl starting materials other than 3-acetoxymethyl compounds may be obtained by the method of Belgian Patent No. 674,255.

Further cephalosporin starting materials which may be used in accordance with the invention may be prepared for example as described in British Patent Specification Nos. 1,012,943; 1,059,562; 1,101,423, and 912,541.

7β - Acylamino - cephalosporin compounds having an etherified hydroxymethyl group at position-3 such as an alkoxymethyl group, especially a methoxymethyl group, may be prepared by the methods described in copending application No. 38493/67 (Serial No. 1241656) filed on even date.

Suitable imide halide forming reagents for use in the process according to the present invention include acid halides (chlorides, bromides and iodides) derived from the phosphorus acids, the preferred compounds being, for example, phosphorus oxychloride or phosphorus pentachloride.

The reaction with the imide halide forming reagent may be carried out in the presence of an organic or inorganic base. Suitable organic bases include tertiary amines such as, for example, triethylamine pyridine or dimethylaniline while calcium carbonate is an example of a suitable inorganic base. Although the base may be omitted the yield of the desired compound may be lower.

The imide halide formation is conveniently effected in solution in an inert organic solvent such as a chlorinated hydrocarbon e.g. methylene chloride. Preferably the imide halide-forming reagent is introduced into the reaction mixture in the form of a solution in an organic solvent.

The imide halide may be converted into the imino ether by reacting with an alcohol, preferably methanol, in the presence of a tertiary amine such as those referred to above. The course of this reaction should be carefully followed, e.g. by thin layer chromatography, to ensure maximum conversion into the desired imino ether. The imino ether may be hydrolyzed or alcoholysed by using water or an alkanol, e.g. methanol, in the presence of a basic or acidic catalyst. Acidic catalysts which may be used include mineral and organic acids such as hydrochloric acid, trifluoroacetic acid, *p*-toluenesulphonic acid, phosphoric acid or formic acid. Suitable basic catalysts include ammonia and salts of weak acids with an alkali metal or alkaline earth metal.

In carrying out the process of the present invention we prefer to use as starting materials compounds in which the 4-position carboxyl group is blocked by esterification with an esterifying group that may easily be split off e.g. by hydrolysis or reduction, if desired, at some later stage. The esterifying group at the 4-position may be retained until the 7β-amino compound obtained has been reacylated. However the esterifying group may be lost during one of the reaction stages.

Alcohol and phenol esterifying groups which may readily be split off include those containing electron-attracting substituents for example sulpho groups and esterified carboxyl groups. These groups may be split off by alkaline reagents but care should be exercised in using these reagents not to cause $\Delta^3 \rightarrow \Delta^2$ isomerisation. Benzyl ester groups at the 4-position may be removed by hydrogenolysis although this may involve catalyst poisoning. A preferred method involves acid hydrolysis and groups which may be removed by acid hydrolysis include the adamantyl group and residues of tertiary butyl alcohol, or of alkanols containing electron donors in the α-position such as acyloxy, alkoxy, halogen, alkylthio, phenyl, alkoxyphenyl, aromatic, heterocyclic or tertiary butyl radicals. These radicals may be derived from alcohols such as *p*-methoxybenzyl alcohol, di-*p*-methoxyphenyl-methanol, triphenylmethanol and diphenylmethanol or a reactive derivative thereof e.g. the bromide or the diazo derivative. Compounds having *inter alia* a diphenylmethoxycarbonyl, a β,β,β-trichloroethoxycarbonyl or a t-butoxy carbonyl group at the 4-position are advantageous because esters of this type do not appear to undergo appreciable $\Delta^3 \rightarrow \Delta^2$ isomerisation under the conditions of the reaction. An alcohol residue which may be readily split off by a reducing agent is that of β,β,β-trichloroethanol which may be removed by Zn/acetic acid; the diphenylmethyl and *t*-butyl groups may be removed by treatment with trifluoroacetic acid and anisole at room temperature.

Other ester groups at the 4-position which can readily be removed include silyl esters.

Although the silyl ester may be formed by reacting the 4-carboxyl group with a silanol

in some cases it may be more convenient to react the 4-carboxyl group with a derivative of a silanol e.g. the corresponding chloride or amine. Thus silyl esters are formed with tetravalent silicon moieties, and the silylating agent conveniently is a silazane of the formula

$$R^4_3Si.NR^4_2,\quad R^4_3Si.NH.SiR^4_3$$
$$R^4_3Si.NH.COR^4,\quad R^4_3Si.NH.CO.NH.SiR^4_3,$$
$$R^4NH.CO.NR^4.SiR^4_3 \text{ or}$$
$$R^4C(OSiR^4_3):NSiR^4_3$$

where the various groups R^4, which can be the same or different, represent hydrogen atoms or alkyl, aryl, or aralkyl groups. Some of these compounds may not be particularly stable under the reaction conditions where R^4 is H for all R^4 groups. It is generally preferred that the R^4 groups of the silazane should be hydrocarbon groups and preferably the hydrocarbon group should be methyl or phenyl as, for example, in hexamethyldisilazane, $(Me_3Si)_2NH$. When preparing the esters on a commercial scale it may be advantageous to employ silyl chlorides such as, for example, Me_3SiCl, in conjunction with a weak base such as, for example, Et_2NH giving silylamines for example $Me_3Si.NEt_2$. The reaction can be followed by measuring the amount of volatile amine or ammonia produced if such compounds are decomposition products. Silazanes which give rise to ammonia or volatile amines are preferred because the base is volatilised under the reaction conditions, thereby avoiding Δ^2 isomerization which might otherwise occur. An inert gas is desirably passed through the reaction mixture to entrain volatile products and keep out moisture.

Silanes such as R^4SiH where R^4 has the above-given meaning are not particularly suitable in this process since in general they are too reactive. Where the silylating agent is a halide, e.g., Me_3SiCl, causing formation of hydrogen halide during silylation, a weak base, e.g. pyridine, is desirably used as acid acceptor. Strong bases may cause isomerisation of the cephalosporin derivative to the corresponding Δ^2 compound.

At least 1 mole of organosilicon compound is used, and an excess of up to 3 or even more moles may be used, to effect silylation. Undiluted organosilicon compounds may be employed as the reaction medium for the silylation or an inert diluent such as a hydrocarbon e.g. benzene, toluene or a xylene, or a halogenated hydrocarbon e.g. chloroform or methylene chloride may be used.

In effecting imide-halide formation on silyl esters advantageous results may be obtained by consideration of the relative proportions of the reactants. Thus a large excess of base may produce unsatisfactory yields. A ratio of silyl ester: imide halide forming component: base of 1:1:4 has been found to be a useful ratio to employ. The 1:1:4 ratio of reactants is particularly effective when the imide halide forming component is phosphorus pentachloride and the base is pyridine.

A further advantage accruing from the use of silyl esters in the process according to the invention is that the esterifying group is removed under mild conditions and hence tends to be removed during one of the reaction stages e.g. during the formation of the imino ether.

The silyl ester group is easily split by treatment with a compound or compounds containing active hydrogen, e.g., water, acidified or basified water, alcohols and phenols.

A wide variety of acyl groups may be removed from 7β-acylamino cephalosporin compounds by the process of the present invention. Again the resulting 7β-amino compounds may be reacylated with a wide variety of acids. Classes of such acyl groups include those classes having the general formulae:—

(i) $R^u(CH_2)_nCO$—where R^u is aryl (carbocyclic or heterocyclic), cycloalkyl, substituted aryl, substituted cycloalkyl, or a non-aromatic heterocyclic group, and n is an integer from 1—4. Examples of this group include phenylacetyl, substituted phenylacetyl, e.g. fluorophenylacetyl, nitrophenylacetyl, acetoxyphenylacetyl, alkanoylphenylacetyl, or hydroxyphenylacetyl; thienyl-2- and -3-acetyl; 4-isoxazolyl- and substituted 4-isoxazolylacetyl and pyridylacetyl. The substituted 4-isoxazolyl group may be a 3-aryl-5-methylisoxazol-4-yl group, the aryl group being e.g. phenyl or halophenyl, e.g., chloro- or bromophenyl. An acyl group of this type is 3-*o*-chloro-phenyl-5-methyl-isoxazol-4-yl-acetyl.

(ii) $C_nH_{2n+1}CO$—where n is an integer from 1—7. This class of group may be straight or branched, and if desired, may be interrupted by an oxygen or sulphur atom or substituted by e.g. a cyano or carboxy group. Examples of such groups include acetyl, hexanoyl, heptanoyl, octanoyl, butylthioacetyl, cyanoacetyl, adipoyl and glutaroyl.

(iii) $C_nH_{2n-1}CH_2CO$—where n- is an integer from 2—7. Such alkenyl groups may be straight or branched and, if desired, may be interrupted by an oxygen or a sulphur atom. Examples of such groups include allylthioacetyl.

(iv) $R^vO.CH_2.CO$—where R^v is aryl (carbocyclic or heterocyclic) cycloalkyl, substituted aryl, substituted cycloalkyl or a non-aromatic heterocyclic group. An example of such a group is phenoxyacetyl.

(v) $R^vSCH_2.CO$—where R^v is as defined above. Examples of such thio groups include S-phenylthioacetyl, S-chlorophenylthioacetyl and S-bromophenylthioacetyl.

(vi) $R^v(CH_2)_nZ(CH_2)_p.CO$—where R^v is as defined above, p is an integer from 1—5, n is an integer from 1—4, and Z is an oxygen or sulphur atom. Examples of such groups include

S-benzylthioacetyl, S-benzylthiopropionyl and S-phenethylthioacetyl.

(vii) R^uCO—where R^u has the meaning defined above. Examples of such groups include benzoyl, substituted benzoyl (e.g. aminobenzoyl), 4-isoxazolyl- and substituted 4-isoxazolyl-carbonyl, and cyclopentanecarbonyl. Where the benzoyl group is substituted the substituents may, for example, be alkyl or alkoxy and may be in the 2- or 2- and 6-positions; an example of such a group is 2,6-dimethoxybenzoyl. Where the group R^u represents a substituted 4-isoxazolyl group, the substituents may be as set out under (i) above. An acyl group of this type is 3 - *o* - chlorophenyl - 5 - methyl - isoxazol - 4 - yl - carbonyl.

(viii) Amino acyl (other than δ-aminadipoyl- or -aminoadipoyl having one or both of the carboxyl and amine groups thereof blocked), for example $R^wCH(NH_2).(CH_2)_nCO$ or $NH_2.Ar(CH_2)_nCO$, where *n* is an integer from 1—10, R^w is a hydrogen atom or an alkyl, aralkyl or carboxyl group or a group as defined under R^v above, and Ar is an arylene group, e.g. *p*-phenylene or 1,4-naphthylene. Examples of such groups are disclosed in British Patent Specification No. 1,054,806. A group of this type is the *p*-aminophenylacetyl group.

(ix)

$$R^x—N(R^x)—CO—$$

where the group R^x, which may be the same or different, are hydrogen atoms or monovalent organic groups, e.g., lower alkyl or halogen substituted lower alkyl.

(x) Substituted glyoxylyl groups of the formula $R^x.COCO$—where R^x is an aliphatic, araliphatic or aromatic group, e.g., a thienyl group, a phenyl group, or a mono-, di- or tri- substituted phenyl group, the substituents being, for example, one or more halogen atoms (F, Cl, Br or I), methoxy groups, methyl groups, or amino groups, or a fused benzene ring. Included in this group are also the α-carbonyl derivatives of the above substituted glyoxylyl groups formed, for example, with hydroxylamine, semicarbazide, thiosemicarbazide, isoniazide or hydrazine.

(xi) α-Substituted carboxylic acid acyl groups, where the α-substituent is an amino, substituted amino [e.g. acylamino or a group obtained by reacting the amino group and/or acylamino group(s) with an aldehyde or ketone e.g. acetone or methyl ethyl ketone], hydroxy, carboxy, esterified carboxy, cyano, halogeno, acyloxy (e.g. formyloxy or lower alkanoyloxy) or etherified hydroxy group. The carboxylic acid may be aliphatic, e.g. an α-substituted paraffinic acid, or araliphatic, e.g., an α-substituted phenylacetic acid.

The process according to the invention is particularly applicable to the removal of 7β-acyl groups which do not possess a functional group requiring blockage. Important acyl groups of this type include thienylacetyl, phenylacetyl, phenoxyacetyl, benzoyl and acetyl.

As mentioned above the compounds of the present invention are useful intermediates in preparing compounds in the cephalosporin series having antibacterial properties. Such compounds may conveniently be prepared by reacylating the 7β-amino group of the N-deacylated cephalosporin compound used as starting material to produce a 7β-acylamino cephalosporin compound other than the 7β-acylamino compound used as starting material in the N-deacylation process. The N-deacylated cephalosporin compound may be employed as the free 4-COOH compound or as an ester with an alcohol or phenol which may readily be split off, e.g. by hydrolysis or reduction, at a later stage of the reaction.

The 7β-amino cephalosporin compounds obtained by the process according to the invention are conveniently recovered as acid addition salts and if desired used as such in the subsequent reacylations, for example as described in the Examples hereinafter. Examples of such acid addition salts are hydronitrates and hydrocarbyl sulphonates, examples of hydrocarbyl sulphonates being alkylbenzene sulphonates, e.g. *p*-toluene sulphonates and lower (C_1—C_8) alkane sulphonates e.g. methane sulphonates. It is advantageous to recover the 7β-amino compound in salt form since high yields of the desired 7β-amino compound can be thus obtained.

In effecting acylations at the 7β-amino group the carboxyl group at the 4-position may be blocked by esterification with diphenylmethanol. Alternatively one may use silyl esters as described above.

Acylating agents which may be used include acid halides (e.g. chlorides and bromides), anhydrides or mixed anhydrides, e.g. with pivalic acid or formed with a haloformate, e.g. a lower alkylhaloformate, or an active ester or azide; alternatively, the acid itself can be used, together with an esterifying agent, e.g. carbonyldiimidazole or a carbodiimide such as N,N′-diethyl-, dipropyl-, or -diisopropyl-carbodiimide, or preferably N,N′-dicyclohexylcarbodiimide.

The N-acylation may be carried out in an aqueous medium with an acid halide, for example in an aqueous solution of a water-miscible ketone such as acetone, or in an aqueous solution of tetrahydrofuran, preferably also in the presence of an acid binding agent for example sodium bicarbonate. The pH is preferably maintained at from 5 to 7 during the reaction, which may be carried out at temperatures of from 0 to 25°C. The acylation may also be carried out in an organic

solvent medium such as ethyl acetate by, for example, simple refluxing.

Alternatively the acylation may be effected with an acid halide or mixed acid anhydride under substantially anhydrous conditions in the liquid phase in an inert Lewis base (preferably one having a tertiary nitrogen atom) having a dielectric constant above 15 and preferably above 30 and containing a hydrogen halide acceptor. The dielectric constant of the base is advantageously within the range of 30—40 and the base is preferably N,N-dimethylformamide or N,N-dimethylacetamide. The reaction may be carried out on the free 4-COOH compound itself on an ester thereof or on an acid addition salt of the 4-COOH compound or ester thereof e.g. the *p*-toluenesulphonate. Advantageously one employs an acid addition salt of said ester under the aforesaid substantially anhydrous conditions. The acid halide is advantageously the chloride or bromide.

Methods of effecting acylation under substantially anhydrous conditions are described more fully in British Patent Specification No. 1,104,937.

The following Examples illustrate the invention. In the Examples the following should be noted:—

System A is descending *n*-propanol: water= 7:3 on Whatman (Registered Trade Mark) No. 1 paper at room temperature.

System B is *n*-butanol: ethanol: water= 4:1:5, equilibrated at room temperature; descending manner with upper phase as developer in equilibrium with lower phase. Whatman No. 3MM paper buffered to pH 6.0 with 0.05 M-sodium dihydrogen phosphate.

System C is ethyl acetate: *n*-butanol: 0.1M-sodium acetate (pH 5.0), equilibrated at 38° descending manner with upper phase as developer in equilibrium with lower phase. No. 1 Whatman paper buffered to pH 5.0 with 0.1 M-sodium acetate.

R_T represents the R_F value divided by that of 3 - acetoxymethyl - 7β - (2' - thienylacetamido)ceph - 3 - em - 4 - carboxylic acid. R_P represents the R_F value divided by that of 3 - acetoxymethyl - 7β - phenylacetamidoceph - 3 - em - 4 - carboxylic acid.

The conditions for electrophoresis are those described by *Cocker et. al., J. Chem. Soc.*, 1965, 5015.

All temperatures are given in degrees Centigrade.

Example 1.

Preparation of 7β - (D - α - amino - α - phenylacetamido) - 3 - methoxymethylceph - 3 - em - 4 - carboxylic acid.

The title compound was prepared via the following reaction scheme (the steps are described in more detail below).

3 - Hydroxymethyl - 7β - (2' - thienylacetamido) - ceph - 3 - em - 4 - carboxylic acid

↓ (a)

Diphenylmethyl 3 - hydroxymethyl - 7β - (2' - thienylacetamido) - ceph - 3 - em - 4 - carboxylate

↓ (b)

Diphenylmethyl 3 - methoxymethyl - 7β - (2' - thienylacetamido) - ceph - 3 - em - 4 - carboxylate

↓ (c)

Diphenlymethyl 7β - amino - 3 - methoxymethylceph - 3 - em - 4 - carboxylate hydrogen *p*-toluenesulphonate

↓ (d)

7β - (D - α - Amino - α - phenylacetamido) - 3 - methoxymethylceph - 3 - em - 4 - carboxylic acid.

a) Diphenylmethyl 3 - hydroxymethyl - 7β - (2' - thienylacetamido)ceph - 3 - em - 4 - carboxylate

3 - Hydroxymethyl - 7β - (2' - thienylacetamido)ceph - 3 - em - 4 - carboxylic acid (500 mg.) was dissolved in dry tetrahydrofuran and treated with a solution of diphenyldiazomethane (300 mg., 1.1 equiv.) in petrol. Nitrogen was evolved slowly, and after 2½ hours the solution was evaporated, the residue dissolved in ethyl acetate, and the solution was washed with bicarbonate solution and re-evaporated. This gave a gum which solidified on trituration with ether (0.5 g.). A sample was recrystallised from methanol, m.p. 164°, $[\alpha]_D^{23} = +25°$ (c, 1.0, dioxane), +22° (c 1.0, tetrahydrofuran). $\lambda\lambda_{max.}$ ethanol 234 nm, $E^{1\%}_{1\,cm.}$ = 255, (ϵ = 13,300), 259 nm, $E^{1\%}_{1\,cm.}$ 151 (ϵ 7,850), $\nu_{max.}$ (bromoform) 3420 (OH), 3280 (NH), 1750 (β-lactam), 1722 cm^{-1} (COOR). (Found, C, 62.2; H, 4.5; N, 5.4; S, 12.1. $C_{27}H_{24}N_2O_5S_2$ requires C, 62.3; H, 4.7; N, 5.4; S, 12.3%) R_f = 0.83 (Kieselgel G plates; ethyl acetate: benzene = 1:2).

(b) Diphenylmethyl 3 - methoxymethyl 7β - (2' - thienylacetamido) - ceph - 3 - em - 4 - carboxylate

Diphenylmethyl 3 - hydroxymethyl - 7β - (2' - thienylacetamido) - ceph - 3 - em - 4 - carboxylate (20 g., 38.4 mmole) was dissolved in dry methylene chloride (1,500 ml.) and treated with boron trifluoride etherate (0.3 ml., 2 mmole.). A solution of diazomethane (from 40 g. nitrosomethylurea) in ether (1,500 ml.) was added at 0°. The solution was kept at room temperature for one hour, when it was filtered through a pad of silica acid, which was finally well washed with ethyl acetate. The solvent was evaporated and the resulting foam dissolved in ethanol (100 ml.) from which the title compound separated as a white crystalline solid (14.3 g., 68%), m.pt. 146—

148° $[\alpha]_D^{25°}=15.6°$ (c 1, tetrahydrofuran), $\lambda\lambda_{max.}$ (ethanol) 235 and 260 nm. (ϵ 14,800 and 9,100 resp). (both shoulders) $\nu_{max.}$ (Nujol) 3295 (—NH), 1786 (β-lactam), 1725, 1230 (—COOR), 1668 and 1646 (—CONH—) cm^{-1} (Found: C, 63.1; H, 4.9; N, 5.1; S, 11.5. $C_{28}H_{26}N_2O_5S_2$ requires: C, 62.9; H, 4.9; N, 5.2; S, 12.0%), N.M.R. ($CDCl_3$) 5.76 ($—CH_2OCH_3$), 6.80 τ ($—CH_2OCH_3$).

(c) Diphenylmethyl 7β - amino - 3 - methoxymethylceph - 3 - em - 4 - carboxylate, hydrogen p - toluenesulphonate

A solution of diphenylmethyl 3 - methoxymethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate (4.0 g., 7.65 mmole.) in methylene chloride (60 ml.) was cooled to −10° and treated with pyridine (7.5 ml. 95 mmole.). A solution of phosphorus pentachloride (4.7 g., 22.6 mmole.) in methylene chloride (70 ml.) was added with stirring over 5 minutes, the temperature being maintained at −10°. The stirring was continued for 30 minutes. Cold methanol (75 ml.) was then added at such a rate that the temperature did not rise above −10°. Stirring was continued for 2½ hours at room temperature. N-hydrochloric acid (105 ml.) was then added and the mixture stirred at room temperature for one hour. The organic layer was separated, washed with aqueous sodium bicarbonate and water, and then evaporated. The residue was dissolved in ethyl acetate (20 ml.) and a solution of p-toluenesulphonic acid (1.45 g., 7.65 mmole.) in ethyl acetate added. The title compound separated as a white crystalline solid which was filtered, washed with ethyl acetate, and dried (3.0 g., 67%). This material could be recrystallised from chloroform/ethyl acetate, m.pt. 150° $[\alpha]_D^{25°}=-8.3°$ (c 1, chloroform), $\lambda_{max.}$ (ethanol) 262 nm. (ϵ 7,359), $\nu_{max.}$ Nujol) 1788 (β-lactam), 1732 and 1230 (—COOR) $cm.^{-1}$ (Found: C, 58.7; H, 5.0; N, 4.6; S, 10.6 $C_{29}H_{30}N_2O_7S_2$ requires: C, 58.9; H, 5.3; N, 4.7; S, 10.8%), N.M.R. ($CDCl_3$) 5.50 and 5.83 (quartet; J=15 c/s, $—CH_2OCH_3$), 6.86 ($—CH_2OCH_3$).

(d) 7β - (D - α - Amino - α - phenylacetamido) - 3 - methoxymethylceph - 3 - em - 4 - carboxylic acid

N - (tert - butoxycarbonyl) - D - phenylglycine (1.85 g., 7.72 mmole.) was dissolved in dry tetrahydrofuran (20 ml.) and the solution cooled to −6°. Triethylamine (1.09 ml., 7.72 mmole.) was added followed by a solution of isobutyl chloroformate (1.06 g. 7.72 mmole.) in dry tetrahydrofuran (4 ml.) at such a rate that the temperature remained below −6°. After stirring for 30 minutes at room temperature, the triethylammonium chloride was filtered off. The filtrate was added to a solution of diphenylmethyl 7β - amino - 3 - methoxymethylceph - 3 - em - 4 - carboxylate hydrogen p - toluene sulphonate (the product of Example 1 (c)) (3.0 g., 5.1 mmole.) in acetonitrile (15 ml.) in dimethylacetamide (5 ml.). After 30 minutes the solvents were removed under reduced pressure and the residue dissolved in ethyl acetate. The solution was extracted with saturated aqueous sodium bicarbonate and water, then dried and the solvent evaporated. The gum was dissolved in a mixture of anisole (3 ml.) and trifluoroacetic acid (12 ml.) and after 5 minutes the solution evaporated under high vacuum. The oil was dissolved in ethyl acetate and the solution poured into a large volume of 60—80° light petroleum. The product was filtered, dried, and suspended in water (200 ml.). The suspension was treated with 'Amberlite' (Registered Trade Mark) LA1 (AcO^-) (20% v/v in ether, 50 ml.) and well shaken; the emulsion which formed was separated by centrifuging. The aqueous layer was washed with ethyl acetate (3 × 40 ml.) and freeze-dried. The white freeze-dried solid (1.4 g., 72%) was crystallised from aqueous propanol, m.pt 245—360° (d) $[\alpha]_D=+100°$ (c 1, water) $\lambda_{max.}$ (water) 260 nm. (ϵ 8,100), $\nu_{max.}$ (Nujol) 1756 (β-lactam) 1512 and 1692 (—CONH—), 1587 ($—COO^-$) $cm.^{-1}$, (Found: C, 52.9, H, 5.1; N, 10.5; S, 8.1. $C_{17}H_{19}N_3O_5S.\frac{1}{2}H_2O$ requires: C, 52.8; H, 5.2; N, 10.9; S, 8.3%). N.M.R. (D_2O) 2.41 (phenyl), 5.71, 6.68 τ ($—CH_2OCH_3$), R_f=0.09 (system B); this substance moved to the anode on electrophoresis at pH 1.9.

Example 2.

7β - (p - Chlorophenylglyoxamido) - 3 - methoxymethylceph - 3 - em - 4 - carboxylic acid

Prepared by acylation of diphenylmethyl 7β - amino - 3 - methoxymethyl - ceph - 3 - em - 4 - carboxylate hydrogen - p - toluene sulphonate (2.5 g., 4.3 mmole.) with the mixed anhydride from p-chlorophenylglyoxylic acid (1.15 g., 6.4 mmole.) and pivaloyl chloride, by the general procedure described in Example 1(d). Yield (1.5 g., 85%), m.pt. 154—156° $[\alpha]_D^{25°}=98.5°$ (c 1, tetrahydrofuran), $\lambda_{max.}$ (ethanol) 264 nm. (ϵ 21, 200), $\nu_{max.}$ (Nujol) 1780 (β-lactam), 1674, 1520 (—CONH—), 1690 (—COOH), 1720 (PhCOCO—) $cm.^{-1}$ (Found: C, 49.1; H, 3.9; N, 6.5; S, 8.0. $C_{17}H_{15}ClN_2O_6S$ 1/4 H_2O requires: C, 49.2; H, 3.8; N, 6.7; S, 7.7%), N.M.R. ($CDCl_3$) 2.54, 1.70 (phenyl), 5.57, 6.63 τ ($—CH_2OCH_3$). R_f 0.43 (system B), 0.65 (system C).

Example 3.

(a) Diphenylmethyl 7β - amino - 3 - ethoxymethylceph - 3 - em - 4 - carboxylate hydrogen p - toluenesulphonate

Diphenylmethyl 3 - hydroxymethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate (10 g., 19.2 mmole.) was dissolved in dry methylene chloride (1000 ml.) and treated with boron trifluoride etherate (0.2

ml., 1.33 mmole.). A solution of diazoethane (from 100 g. nitrosylethylurea) in 60—80° light petroleum (1000 ml.). was added at room temperature. After 30 minutes the solution was filtered through silica acid and the silica acid washed with ethyl acetate (3 × 50 ml.). The solvents were evaporated and the residual gum chromatographed on a silicic acid column (6.5 × 25 cms) in ethyl acetate: benzene = 1:9. Those fractions containing the major constituent in the gum (R_f 0.7 in ethyl acetate: benzene = 1:5 on silica gel G) were bulked and evaporated to give a gum (4.1 g., 39%) which was diphenylmethyl 3 - ethoxymethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate. The 7-side chain was removed and the hydrogen-*p*-toluene sulphonate formed in a manner similar to that described in Example 1(c). Yield of the title compound (1.42 g., 12.5% based on the hydroxymethyl compound), m.pt. 154°, $[\alpha]_D^{25°} = -7.1°$ (c 1, $CHCl_3$) $\lambda_{max.}$ (ethanol) 262 nm. (ε 7,400), $\nu_{max.}$ (Nujol) 1782 (β-lactam), 1220, 1712 (—COOR), 1175 (SO_3^-) cm.$^{-1}$ (Found: C, 59.6; H, 5.3; N, 4.5; S, 10.0. $C_{30}H_{32}N_2O_7S_2$. $1/2H_2O$ requires: C, 59.5; H, 5.5; N, 4.6; S, 10.6), N.M.R. ($CDCl_3$) 5.44 and 5.83 (quartet: J = 16 c/s, $—CH_2OCH_2CH_3$), 6.78, 8.95 τ ($—CH_2OCH_2CH_3$).

(b) 7β - (D - α - Amino - α - phenylacetamido) - 3 - ethoxymethylceph - 3 - em - 4 - carboxylic acid

Prepared by acylation of diphenylmethyl - 7β - amino - 3 - ethoxymethylceph - 3 - em - 4 - carboxylate hydrogen *p* - toluenesulphonate (2.0 g., 3.34 mmole.) with the mixed anhydride from N - (tert - butoxycarbonyl) - D - phenylglycine (1.21 g., 4.8 mmole.) and isobutylchloroformate, by the general method described in Example 1 (d). Yield (1.1 g., 84%), m.pt 183—230° (d) $[\alpha]_D^{22°} = 74.8°$ (c 1, H_2O), $\lambda_{max.}$ (water) 260 nm (ε 7,350), $\nu_{max.}$ (Nujol) 1775 (β-lactam), 1698, 1560 (—CONH—), 1630 (COO^-) cm.$^{-1}$, N.M.R. (D_2O) 2.45 (phenyl), 5.80, 6.50, 8.85 τ ($—CH_2OCH_2CH_3$), R_f 0.66 (system A), 0.13 (System B).

Example 4.

(a) Diphenylmethyl 3 - chloromethyl - 7β - (2′ - thienylacetamido)ceph - 3 - em - 4 - carboxylate

Diphenylmethyl 3 - hydroxymethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate (5.2 g., 10 mmole.) and pyridine (4 ml:, 40 mmole.) in dry tetrahydrofuran (75 ml.) were added dropwise at 20° to a solution of thionyl chloride (2.38 g., 1.45 ml; 20 mmole.) in dry tetrahydrofuran (25 ml.) during one hour. After 15 minutes the mixture was poured into brine and the product extracted into ethyl acetate; the organic extract was dried and concentrated. The concentrate was added dropwise to petroleum ether (b.p. 40—60°) and the solid product (3.9 g., 73%) collected. A sample was crystallised from ethanol m.p. 125—133° (decomp) $[\alpha]_D^{23} = 6.5°$ (c 1.0, tetrahydrofuran) $\lambda\lambda_{max.}$ (ethanol) 235 nm. (ε 13,200), 266 nm. (ε 8,000), $\nu_{max.}$ (bromoform) 3390 (NH), 1785 (β-lactam), 1725 (COOR), 1682 and 1510 cm^{-1} (CONH), τ($CDCl_3$) 5.63. 3-methylene group singlet. (Found: C, 60.7; H, 4.7; N, 4.7; S, 11.7; Cl, 6.2 $C_{27}H_{23}N_2O_4S_2Cl$ requires, C, 60.2; H, 4.3; N, 5.2; S, 11.9; Cl, 6.6%), R_f = 0.47 (silica plates benzene: ethyl acetate = 5:1).

(b) Diphenylmethyl 7β - amino - 3 - n - propoxymethylceph - 3 - em - 4 - carboxylate hydrogen *p* - toluenesulphonate

A solution of diphenylmethyl 3 - chloromethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate (1.5 g., 2.8 mmole.) in acetone (80 ml.) was treated with a solution of sodium iodide (0.45 g., 3.0 mmole.) in acetone (15 ml.). The solution was allowed to stand in the dark for 1 hour at room temperature. The solution was filtered and poured into water. The mixture was extracted with ether (3 × 15 ml.) and the ether extracts dried over magnesium sulphate and evaporated. The gum was dissolved in *n*-propanol (50 ml.) and treated with mercuric perchlorate hydrate (4 g., 9.6 mmole.). After 5 minutes the black precipitate was filtered on Kieselguhr. The filtrate was evaporated under reduced pressure and the residue was dissolved in ethyl acetate, filtered, washed with aqueous sodium bicarbonate and water, dried, and evaporated. The resulting gum was chromatographed on a silicic acid column (4.5 × 25 cms.) in benzene: ethyl acetate = 9:1. The fractions containing the *n*-propoxymethyl compound (R_f *ca.* 0.7, ethyl acetate: benzene = 1.5, on silica gel G) were bulked and evaporated (0.6 g., Yield *ca.* 38%). The 7-side chain was removed and the hydrogen-*p*-toluene sulphonate formed in a manner similar to that described in Example 1(c). Yield of the title compound (85 mg., 5% from the 3-chloromethyl compound). This material crystallised as colourless needles from chloroform-ethyl acetate, m.pt 155—9° (d) $[\alpha]_D^{21°} = -7°$ (c 1, $CHCl_3$), $\lambda_{max.}$ (ethanol) 263 nm. (ε 7,000), $\nu_{max.}$ (Nujol) 1788 (β-lactam), 1710 (—COOR), 1185 (SO_3^-) cm.$^{-1}$, N.M.R. ($CDCl_3$) 5.42 and 5.79 ($—CH_2OCH_2CH_2CH_3$), 6.80, 8.58, 9.18 τ ($—CH_2OCH_2CH_2CH_3$). (Found C, 60.9; H, 5.7; N, 4.2; S, 10.1; $C_{31}H_{34}N_2O_7S_2$ requires C, 61.0; H, 5.6; N, 4.6; S, 10.5.)

Example 5.

(a) Diphenylmethyl 3 - benzoyloxymethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate

Pyridine (1.58 g., 1.58 ml., 20 mmole.) was added dropwise at room temperature to a solu-

tion of diphenylmethyl 3 - hydroxymethyl - 7β - (2' - thienylacetamido)ceph - 3 - em - 4 - carboxylate (2.08 g., 4 mmole.) (prepared as described in Example 1(a) in dry tetrahydrofuran (50 ml.) containing benzoyl chloride (2.88 g., 2.36 ml., 20 mmole.). The mixture was stirred for 45 minutes, filtered, and evaporated to dryness. The residue was partitioned between ethyl acetate and N-sodium bicarbonate solution, and the organic layer washed with dilute sodium bicarbonate solution, hydrochloric acid and finally brine. The dry solution was evaporated to give a solid (2.389 g., 96%) which was washed with ether, R_f=0.78 (Kieselgel G, ethyl acetate: benzene=1:2), R_f =0.62 (Kieselgel G, ethyl acetate: benzene= 1:5).

(b) Diphenylmethyl 7β - amino - 3 - benzoyloxymethylceph - 3 - em - 4 - carboxylate hydrogen *p* - toluene sulphonate

Dry pyridine (16 ml., 192 mmole.) was added to a solution of diphenylmethyl 3 - benzoyloxymethyl - 7β - (2' - thienylacetamido)ceph - 3 - em - 4 - carboxylate (10 g., 16 mmole.) in dry methylene chloride (125 ml.) at −10°. The solution was stirred while a solution of phosphorus pentachloride (10 g., 48 mmole.) in methylene chloride (150 ml.) was added during 5 minutes: stirring and cooling were continued for 30 minutes. Methanol (160 ml.) which had been cooled in ice was added at such a rate that the temperature did not exceed −10° and the reaction was then allowed to progress at −10° for 30 minutes and for a further 90 minutes at room temperature. The mixture was cooled to 0° and N-hydrochloric acid (225 ml.) added; stirring was carried out for 90 minutes. The methylene chloride layer was separated, washed with sodium bicarbonate solution and brine, and dried over magnesium sulphate. Evaporation gave a gum which was dissolved in ethyl acetate (100 ml.); a concentrated solution of toluene *p*-sulphonic acid (3.07 g., 16 mmole.) in ethyl acetate was added and the mixture kept at 0° for 1 hour. The product (8.9 g., 83%) was filtered off, washed with acetone, and dried. m.p. 150—151° (decomp) $[\alpha]_D^{23}$= 12.6° (c 1.0, dimethylsulphoxide), $\lambda\lambda_{max.}$ (ethanol) 221—222 nm. (ε 32,000), 263 nm (ε 8,000), $\nu_{max.}$ (Nujol) 1793 (β-lactam), 1722 (COOR), 710 and 680 cm.$^{-1}$ (Ph). N.M.R. spectrum (dimethylsulphoxide); 9 aromatic protons at 1.9—3.1 (Found, C, 61.6; H, 4.9; N, 3.8; S, 9.2 $C_{35}H_{32}N_2O_8S_2,1/2H_2O$ requires C, 61.7; H, 4.9; N, 4.1; S, 9.4%) R_f=0.3 (Kieselgel G plates; ethyl acetate: benzene= 1:5). At pH 1.9 and at pH 7.0 the product, on electrophoresis, bears a net partial positive charge.

Example 6.

(a) Diphenylmethyl 3 - acetoxymethyl - 7β - valeramido - ceph - 3 - em - 4 - carboxylate

Orange mercuric oxide (3.25 g., 0.015 mole.) and benzophenone hydrazone (2.95 g., 0.015 mole.) in petroleum-ether (100 ml.; b.p. 40—60°) were stirred in the dark for 6 hours at room temperature. The mixture was filtered and the filtrate added to a solution of 3 - acetoxymethyl - 7β - valeramido - ceph - 3 - em - 4 - carboxylic acid (3.56 g., 0.01 mole.) in dioxan (100 ml.). The solution was allowed to stand overnight, then the solvents were evaporated off (rotary evaporator, bath 30°), leaving a deep red syrup. The syrup was dissolved in ethyl acetate (200 ml.) and the solution washed with 2N-hydrochloric acid (200 ml.), 3%-sodium bicarbonate (200 ml.), water 200 ml.), and brine (200 ml.), and then dried (magnesium sulphate). Evaporation of the dried solution left a yellow syrup (6.20 g.), showing a major spot R_f 0.45, and several minor spots on thin-layer chromatography (silica gel GF_{254} as adsorbent; ethyl acetate-benzene, 1:4, as developing solvent; UV detection). The syrup was dissolved in benzene (25 ml.), and petroleum-ether (50 ml.; b.p. 40—60°) was added slowly to the rapidly stirred solution, precipitating a cream powder. This was collected by filtration washed with petroleum ether-benzene (2:1; 20 ml.) and petroleum ether (25 ml.), then dried *in vacuo*, giving *diphenylmethyl 3 - acetoxymethyl - 7β - valeramido - ceph - 3 - em - 4 - carboxylate* (5.22 g., 85%, R_f 0.45 (by thin-layer chromatography as before), $[\alpha]_D^{21}$ + 124° (c=1.02, dioxan), $\lambda_{max.}$ (ethanol) 263—264 nm. (ε 8,300), $\nu_{max.}$ ($CHBr_3$) 1788 (β-lactam), 1730 (ester), 1688 and 1508 cm.$^{-1}$ (amide); τ ($CDCl_3$) 2.63 (10-proton singlet; Ph_2C—), 3.03 (1-proton singlet; $Ph_2CH.O_2C$), 3.50 (1 proton doublet, J 8.5 c/sec.; CONH), 4.12 (1-proton double doublet, J. 8.5, 5 c/sec.; C—7 H), 5.03 (1-proton doublet, J 5 c/sec.; C—6 H), 4.91, 5.24 [2 1-proton doublets (branches of quartet), J 14 c/sec.; C—3 CH_2], 6.38, 6.72 [2 1-proton doublets (branches of a quartet), J 18 c/sec.; C—2 H_2], 7.73 (2-proton triplet, J 7 c/sec.; —CH_2CH_2CONH), 7.97 (3-proton singlet; CH_3CO), 8.00—8.90 (4-proton multiplets: $CH_3CH_2CH_2CH_2$CONH), 9.11 (3-proton triplet, J 6 c/sec.; CH_3CH_2—). (Found: C, 64.6; H, 5.8; N, 5.1; S, 6.0. $C_{28}H_{30}N_2O_6S$ requires C, 64.4; H, 5.8; N, 5.4; S, 6.1%).

(b) Conversion of Diphenylmethyl 3 - acetoxymethyl - 7β - valeramido - ceph - 3 - em - 4 - carboxylate into 3 - acetoxymethyl - 7β - aminoceph - 3 - em - 4 - carboxylic acid

A solution of diphenylmethyl 3 - acetoxymethyl - 7β - valeramido - ceph - 3 - em - 4 - carboxylate (2.61 g., 0.005 mole) in methylene chloride (40 ml.) and pyridine (5 ml. was cooled to −20°, and rapidly stirred while a cold solution of phosphorus pentachloride (3.12 g., 0.015 mole.) in methylene chloride

(50 ml.) was run in over 2 min. The solution (later suspension) was stirred at −10° for 30 min., ice-cold methanol (50 ml.) was added, and the solution stirred for a further 30 min. at −10°, then at room temperature for 1 hr. The solution was cooled to 0°, and ice-cold 1N-hydrochloric acid (70 ml.) was added and the two-phase system was stirred and allowed to warm to room temperature over 45 min. A solution of tripotassium phosphate (7.5 g.) in water (15 ml.) was added, and the pH adjusted to 8.0 by the addition of 2N-sodium hydroxide (57 ml.). The methylene chloride layer was collected and combined with further methylene chloride extracts (2 × 50 ml). of the aqueous layer, the total organic extract washed with water (50 ml.) and brine (50 ml.) then dried (magnesium sulphate). Partial evaporation of the dried solution (rotary evaporator, then oil pump for 1 hr.) left a brown oil (3.54 g.). This oil was diluted with anisole (3 ml.) and the container cooled (ice-water bath) while trifluoroacetic acid (15 ml.) was added. The mixture was kept at 0° until visible reaction ceased, and was then kept at room temperature for 15 min. The mixture was poured into ice-cold methanol (120 ml.); simultaneously tri-n-butylamine (48 ml.) was added to bring the pH to 3.5. The reaction mixture was kept near 0° throughout the additions (ice-salt-water bath), then refrigerated overnight. The precipitate was collected by filtration, washed with methanol (10 ml.) and ether (10 ml.) and dried in vacuo, giving 3 - acetoxymethyl - 7β - amino - ceph - 3 - em - 4 - carboxylic acid (1.02 g., 75%), $\lambda_{max.}$ (pH 6 buffer) 264 nm. (ε 7,750), identified with authentic material by paper chromatography and infrared and proton magnetic resonance spectra.

Example 7.

(a) 3 - Methyl - 7β - phenylacetamido - ceph - 3 - em - 4 - carboxylic acid

7β - amino - 3 - methylceph - 3 - em - 4 - carboxylic acid (2.0 g., 9.35 mmole.) was dissolved in 5% aqueous sodium hydrogen carbonate solution (40 ml., 23.8 mmole.) and acetone (25 ml.) was added. The solution was stirred at 23° while redistilled phenylacetyl chloride (1.5 ml., 11.7 mmole.) in acetone (15 ml.) was added during 10 minutes. The reaction mixture was stirred for a further 20 minutes when ethyl acetate (40 ml.) and 6N-hydrochloric acid (10 ml.) were added with stirring. The aqueous layer was separated and further extracted with ethyl acetate (2 × 25 ml.). The ethyl acetate extracts were combined, stirred for 10 minutes with charcoal (1 g.), dried over anhydrous sodium sulphate (10 g.), filtered and evaporated to low volume to give a crystalline deposit. The slurry was stored at 0° for one hour and its precipitate was collected, washed with a mixture of ethyl acetate and diethyl ether and dried at 40° *in vacuo* to give the *title acid* (1.73 g., 55.5%), $[\alpha]_D$ + 206° (C 0.5; 0.2M pH 7 phosphate buffer), $\lambda_{max.}$ (pH 7) 261 nm. (ε 8,250) (Found: C, 57.2; H, 4.8; N, 8.8; S, 9.75. $C_{16}H_{16}O_4N_2S$ (332.4) requires C, 57.8; H, 4.85; N, 8.45; S, 9.65%), R_f 0.775 (n-propanol-water 7:3).

(b) Diphenylmethyl 3 - methyl - 7β - phenylacetamidoceph - 3 - em - 4 - carboxylate

A solution of 3 - methyl - 7β - phenylacetamidoceph - 3 - em - 4 - carboxylic acid (1.00 g., 3.01 mmole.) in dioxan (15 ml.) was treated with a solution of diphenyldiazomethane (*ca* 1 equiv.) in petroleum ether (b.p. 40—60°, 5 ml.). The mixture was stored overnight and the resulting pink gel was stirred and triturated with ether (100 ml.) to give the title ester (1.42 g., 95%), $[\alpha]_D^{18}$ + 15.5° (C 1.00; chloroform), $\lambda_{max.}$ 258 nm. (ε 7,080), $\nu_{max.}$ ($CHBr_3$) 1770 (β-lactam) and 1715 $cm.^{-1}$ (—CO_2R), n.m.r. ($CDCl_3$ + Me_2SO-d_6) τ 7.82 (3-CH_3). (Found: C, 69.1; H, 5.5; N, 5.4; S, 6.2. $C_{29}H_{26}N_2O_4S$ (498.6 requires C, 69.85; H, 5.3; N, 5.6; S, 6.4%).

(c) Diphenylmethyl 7β - amino - 3 - methylceph - 3 - em - 4 - carboxylate hydrogen *p* - toluenesulphonate

The 7-side chain was removed and the hydrogen - *p* - toluene sulphonate formed in a manner similar to that described in Example 1(c) from diphenylmethyl 3 - methyl - 7β - phenylacetamidoceph - 3 - em - 4 - carboxylate (1.5 g., 3 mmole.). Yield of the crude product (1.14 g., 66%). Trituration with ethyl acetate gave the title compound, $\lambda_{max.}$ (ethanol) 258 nm. (ε 6,750), $\nu_{max.}$ ($CHBr_3$ 1772 (β-lactam), 1713 $cm.^{-1}$ (—CO_2R), N.M.R. ($CDCl_3$) 7.82 τ (3-CH_3) (Found C, 59.0; H, 5.0; N, 4.7; $C_{28}H_{28}N_2O_6S_2$ H_2O requires C, 58.9; H, 5.3; N, 4.9%).

Example 8.

(a) Diphenylmethyl 3 - acetoxymethyl - 7β - (4′ - diphenylmethoxycarbonylbutanamido) - ceph - 3 - em - 4 - carboxylate

Benzophenone hydrazone (26 g., 0.133 mole.), anhydrous sodium sulphate (30 g.), anhydrous ether (400 ml.), saturated ethanolic potassium hydroxide (10 ml.) and yellow mercuric oxide (70 g.) were stirred in the dark for 90 minutes at room temperature. The mixture was filtered, the solid washed well with ether and the combined filtrate and washes concentrated under reduced pressure at room temperature to a dark red oil. This was dissolved in petroleum ether (40—60°, 200 mls.) in which it was completely soluble and added to a solution of 3 - acetoxymethyl - 7β - (4′ - carboxybutan - amido) - ceph - 3 - em - 4 - carboxylic acid (prepared by acylation of 3 - acetoxymethyl - 7β - aminoceph - 3 - em - 4 - carboxylic acid with glutaric anhydride) (14.54 g., 37.6 mmoles.) in dioxan (500 ml.). The mixture was allowed to stand for 16 hours

at room temperature in the dark and concentrated under reduced pressure at room temperature to a red oil. The oil was dissolved in ethyl acetate (300 ml.) and the solution washed with hydrochloric acid (2N; 300 ml.), 3% sodium bicarbonate solution (300 ml.) water (300 ml.) and brine (300 ml.). After the solution had been dried over magnesium sulphate it was concentrated under reduced pressure to give a pale yellow solid. This was dissolved in benzene (200 ml.) and petroleum ether (40—60°; *ca.* 400 ml.) added slowly until the mixture became opalescent, when it was seeded, stirred and allowed to crystallise. Further petroleum ether (40—60°; to a total of 1.0 l.) was added and the crystallisation completed by refrigeration for two days. The solid was harvested by vacuum filtration, washed well with petroleum ether (40—60°) and dried *in vacuo* at 40° overnight to give the title compound (25.32 g., 93.6%), m.p. 125—7; $[\alpha]_D$ + 16.2° (c=0.99, dioxan); TLC, R_f 0.69 and trace impurity on base-line (silica gel as adsorbent; ethyl acetate/benzene 1:2 as developing agent); $E^{1\%}_{1\,cm.}$ (ethanol) $\lambda_{max.}$ 265 nm (ϵ 8,200); Found: C, 67.5%; H, 5.4%; N, 3.7%; S, 4.53%. Calc. for $C_{41}H_{38}N_2O_8S$ C, 68.5%; H, 5.3%; N, 3.9%; S, 4.5%.

(b) 3 - Acetoxymethyl - 7β - aminoceph - 3 - em - 4 - carboxylic acid

A solution of diphenylmethyl 3 - acetoxymethyl - 7β - (4′ - diphenylmethoxy-carbonylbutanamido) - ceph - 3 - em - 4 - carboxylate (3.54 g., 5 mmoles.) in methylene chloride (dried over P_2O_5; 132 ml.) and pyridine (5.1 ml., ~64 mmoles.) was cooled to −20 and a cold solution of phosphorus pentachloride (3.2 g., 15 mmoles.) in dry methylene chloride (57 ml.) added over 3 minutes during which the temperature rose to −14°. The reaction mixture was stirred for 45 minutes at −10° when methanol (33 ml.) pre-cooled to −20° was added over 30 secs. Stirring was continued for 30 minutes at −10°, then for 1 hour at 20° during which period the pale yellow reaction mixture lightened in colour. Ice-cold hydrochloric acid (N, 110 ml.) was added and the mixture stirred vigorously for 45 minutes at 20°. A solution of tripotassium phosphate (4.16 g.) in water (8.4 ml.) was added, followed by sodium hydroxide solution (2N, ~112 ml.) until pH 8.0 was reached. The methylene chloride layer was separated and the aqueous layer re-extracted with methylene chloride (2 × 25 ml.). The combined methylene chloride extracts were washed with water (25 ml.) and brine (25 ml.) then dried over magnesium sulphate (65 g.) before concentration under reduced pressure to an oil (5.44 g.). Anisole (2.5 ml.) was added, the mixture stirred and cooled to −15° and trifluoroacetic acid (7.28 ml.) added dropwise over 7 minutes. The temperature was raised to 25° and the mixture stirred for 30 minutes. It was then run into methanol (88 ml.) simultaneously with triethylamine (*ca* 11 ml.) at such a rate that the pH was always on the acid side of the final target of 3.5 and the temperature was −10°. The mixture was refrigerated overnight, the precipitate collected by filtration, washed with methanol (2 × 5 ml.), methylene chloride (2 × 5 ml.) and ether (2 × 5 ml.), dried *in vacuo* at 40° to yield the title compound (0.940 g., 69.0%); $[\alpha]_D$ + 116.9° (c=1.0, 0.5N $NaHCO_3$ solution) $E^{1\%}_{1\,cm.}$ $\lambda_{max.}$ 263 mμ, ϵ 8,550 (0.1N $NaHCO_3$). The pH of the mother liquors was found to be less than 3.5 and on adjustment a further quantity of 3 - acetoxymethyl - 7β - aminoceph - 3 - em - 4 - carboxylic acid was obtained, (0.135 g., 9.9%), $E^{1\%}_{1\,cm.}$ $\lambda_{max.}$ 267 mμ, ϵ 8,180.

Example 9.

Preparation of 7β - (D - α - amino - α - phenylacetamido) - 3 - (2′ - oxocyclohexyl) - methyl - ceph - 3 - em - 4 - carboxylic acid

The title compound was prepared via the following reaction scheme (the steps are described in more detail below)

Diphenylmethyl 3 - chloromethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate
↓ (a)
Diphenylmethyl 3 - iodomethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate
↓ (b)
Diphenylmethyl 3 - (2′ - oxocyclohexyl)-methyl - 7β - (2″ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate
↓ (c)
Diphenylmethyl 7β - amino - 3 - (2′ - oxocyclohexyl)methyl - ceph - 3 - em - 4 - carboxylate hydrogen *p* - toluenesulphonate
↓ (d)
7β - (D - α - Amino - α - phenylacetamido) - 3 - (2′ - oxocyclohexyl)methylceph - 3 - em - 4 - carboxylic acid.

(a) Diphenylmethyl 3 - iodomethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate

Diphenylmethyl 3 - chloromethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate (7.858 g., 14.5 mmole.) was dissolved in acetone and reacted with sodium iodide (7.8 g., 50 mmole.) in acetone (100 ml.) in the dark for 90 minutes. At the end of this time the solution was filtered and poured into water (750 ml.) containing sodium chloride and sodium thiosulphate. The oil was extracted with ether (4 ×), and the organic layer washed

once with water and twice with brine, and dried and evaporated to a foam, which was crystallised from ethyl acetate (16 ml.) to give the title compound. The mother liquors were washed with sodium thiosulphate, water and brine, and were dried and evaporated to a foam (2.75 g., impure title compound, approx. 30% yield). Yield of crystalline material 60%, m.p. 155—161°C (decomp.), $[\alpha]_D^{28}$ — 86.3° (c 0.73; tetrahydrofuran), $\lambda_{max.}$ (ethanol) 290 nm. (ε 8,400), $\nu_{max.}$ (Nujol) 1773 (β-lactam), 1717 (CO_2R), and 1670 and 1520 $cm.^{-1}$ (—CONH—), N.M.R. (deuterochloroform) τ 5.70 (2-proton broad singlet) (CH_2I) (Found: C, 51.6; H, 3.7; I, 19.7; N, 4.3; S, 10.2. $C_{37}H_{23}IN_2O_4S_2$ requires C, 51.3; H, 3.7; I, 20.1; N, 4.4; S, 10.2), R_f 0.65 Kieselgel G, benzene-ethylacetate=5:1) (T.L.C.).

(b) Diphenylmethyl 3 - (2′ - oxocyclohexyl)-methyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate

A solution of diphenylmethyl 3 - iodomethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate (8 g., 12.7 mmole.) in dry benzene was treated with N - (cyclohex - 1 - enyl) - pyrrolidine (4 g., 26.2 mmole.) and refluxed for 5 minutes. The solution was then cooled and a mixture of 2N - hydrochloric acid (20 ml.) and acetone (80 ml.) added. The suspension was well shaken until all the oil had dissolved and then ethyl acetate (300 ml.) and water (100 ml.) added. After shaking, the aqueous layer was separated and discarded and the organic layer washed with (a) aqueous sodium thiosulphate, (b) aqueous sodium bicarbonate, and (c) water, and then dried and evaporated. The foam was dissolved in ethyl acetate (25 ml.) and after 15 minutes the title compound filtered off, washed with ethanol and dried (4.5 g., 59%). This material crystallised from ethanol as colourless prisms, m.p. 167—170°, $[\alpha]_D^{23}$ + 8.5° (C 1, tetrahydrofuran), $\lambda_{inf.}$ (ethanol) 260 nm (ε 7,500), $\nu_{max.}$ ($CHBr_3$) 1780 (β-lactam), 1720 (COOR), 1702 (>=0), and 1682 and 1512 (CONH—) $cm.^{-1}$, N.M.R. ($CDCl_3$) 7.3. —9.0 τ (protons in cyclohexanone ring) (Found: C, 65.4; H, 5.4; N, 4.5; S, 10.7. $C_{33}H_{32}N_2O_5S_2I/4H_2O$ requires C, 65.6; H, 5.4; N, 4.6; S, 10.6%).

(c) Diphenylmethyl 7β - amino - 3 - (2′ - oxocyclohexyl)methylceph - 3 - em - 4 - carboxylate, hydrogen p - toluenesulphonate

A solution of diphenylmethyl 3 - (2′ - oxocyclohexyl)methyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate (2.25 g., 3.75 mmole.) and pyridine (3.75 ml., 4.75 mmole.) in methylene chloride (30 ml.) was cooled to —10° and treated with a solution of phosphorus pentachloride (2.35 g., 11.3 mmole.) in methylene dichloride (35 ml.) over 5 minutes. The solution was stirred at —10° for 30 minutes. Methanol (37.5 ml.) was added at such a rate that the temperature did not rise above —10°, and when it had all been added, the temperature was allowed to rise to room temperature. After five hours standing the solution was cooled to —10° and treated with water (50 ml.), with vigorous stirring. Stirring was continued at room temperature for 30 minutes. The organic layer was separated and washed successively with dilute acetic acid, aqueous sodium bicarbonate and water, and then dried and evaporated. The gum was dissolved in ethyl acetate (20 ml.) and ether (50 ml.) and treated with p-toluenesulphonic acid (0.73 g., 7.7 mmole.) in ethyl acetate (30 ml.). The title compound separated as colourless prisms (0.80 g., 33%). This material could be recrystallised from chloroform/ethyl acetate, m.p. 162—166° (d), $[\alpha]_D$ — 0.9° (C 1, methylene chloride: methanol= 4:1 (v/v)), $\lambda_{max.}$ (EtOH) 259 nm (ε 6,900, $\nu_{max.}$ (Nujol) 1780 (β-lactam), 1718 (COOR), and 1700 (>=0) $cm.^{-1}$, N.M.R. (in $(CD_3)_2SO$), 7.30 (protons in cyclohexanone ring) 7.5—8.9 τ (protons in cyclohexanone ring), (Found: C, 62.3; H, 5.7; N, 3.9; S, 9.4. $C_{34}H_{36}N_2O_7S_2.\frac{1}{2}H_2O$ requires C, 62.1; H, 5.7; N, 4.3; S, 9.7%).

(d) 7β - (D - α - Amino - α - phenylacetamido) - 3 - (2′ - oxocyclohexyl)methylceph - 3 - em - 4 - carboxylic acid

A solution of N - (t - butoxycarbonyl) - D - phenylglycine (0.92 g., 3.35 m.mole.) in dry tetrahydrofuran (10 ml.) at —6° was treated with triethylamine (0.54 ml., 3.35 mmole.) and then with a solution of isobutylchloroformate (0.53 g., 3.36 mmole.) in dry tetrahydrofuran (4 ml.), at such a rate that the temperature did not rise above —6°. After 30 minutes stirring at room temperature the triethylammonium chloride was filtered off. The filtrate was added to a solution of diphenylmethyl 7β - amino 3 - (2′ - oxo - cyclohexyl) - methylceph - 3 - em - 4 - carboxylate, hydrogen - p - toluenesulphonate (1.5 g., 2.55 mmole.) in acetonitrile (8 ml.) and N,N - dimethylacetamide (4 ml.). After 60 minutes the solvents were removed under reduced pressure. A solution of the residue in ethyl acetate was washed with aqueous sodium bicarbonate and water and then dried and the solvent evaporated. The gum was treated with anisole (3 ml.) and trifluoroacetic acid (12 ml.), and after 5 minutes the reagents removed under vacuum. The oil was suspended in water (100 ml.) and treated with 10%-Amberlite LA1 resin (OAc^-) in ether (50 ml.). After shaking, the aqueous layer was separated and washed with ethyl acetate (4 × 50 ml.) and then freeze-dried to give a white solid (700 mg., 68%), m.p. 150—210°, $\lambda_{max.}$ (H_2O) 261 nm. (ε 6,600), $\nu_{max.}$ (Nujol) 1766 (β-lactam), 1700 (>=0), 1680 and 1530 (—CONH—), and 1620 (—COO^-) $cm.^{-1}$, N.M.R. (in

$(CD_3)_2SO$) 2.52 (phenyl) 7.0—9.0 τ (protons in cyclohexane ring), R_f 0.17 (system B), 0.06 (system C). Electrophoresis at pH 1.9 gives 2 spots, both giving colours with ninhydrin. The faster, which does not absorb ultraviolet light, corresponds in this behaviour with α-phenylglycine. The major fraction absorbs U.V. light.

Example 10.

Preparation of 7β - (D - α - amino - α - phenylacetamido) - 3 - isopropoxymethylceph - 3 - em - 4 - carboxylic acid

The title compound was prepared via the following reaction scheme (the steps are described in more detail below)

Diphenylmethyl 3 - hydroxymethyl - 7β - 2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylate

↓ (a)

Diphenylmethyl 3 - dichloroacetoxymethyl - 7β - (2′ - thienyl acetamido) - ceph 3 - em - 4- carboxylate

↓ (b)

3 - Dichloroacetoxymethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylic acid

↓ (c)

Diphenylmethyl 7β - amino - 3 - isopropoxymethylceph - 3 - em - 4 - carboxylate hydrogen p - toluenesulphonate

↓ (d)

7β - (D - α - Amino - α - phenylacetamido) - 3 - isopropoxymethyl - ceph - 3 - em - 4 - carboxylic acid.

(a) Diphenylmethyl 3 - dichloroacetoxymethyl - 7β - (2′ - thienylacetamido)-ceph - 3 - em - 4 - corboxylate

Diphenylmethyl 3 - hydroxymethyl - 7β - (2′ - thienylacetamido)ceph - 3 - em - 4 - carboxylate (2.08 g., 4 mmole.) and pyridine (1.58 ml.; 20 mmole.) were dissolved in dry tetrahydrofuran (100 ml.) and cooled to −20°. Dichloroacetyl chloride (2.95 g; 1.96 ml; 20 mmole.) in dry tetrahydrofuran (5 ml) was added dropwise. 15 minutes after the addition the mixture was filtered and evaporated, and the residue was partitioned between ethyl acetate and sodium bicarbonate solution. The organic layer was washed with brine, dried and taken to small volume; this solution was then added dropwise to petrol to give a white solid (2.1 g; 85%), m.p. > 60° (softens), $[\alpha]_D^{23}=+17.5°$ (c 1.14, dioxan), $\lambda\lambda_{max.}$ (ethanol) 236 nm (ϵ=13,300), 259 nm (ϵ=7,600), $\nu_{max.}$ ($CHBr_3$) 1783 (β-lactam), 1760 (CO_2CHCl_2), 1725 (COOR), 1680 and 1510 cm^{-1} (CONH). N.M.R. spectrum ($CDCl_3$) —COC*H*Cl_2 4.13 τ R_f=0.37 (Kieselgel G plate; benzene: ethyl acetate=5:1).

(b) 3 - Dichloroacetoxymethyl - 7β - (2′ - thienylacetamido)ceph - 3 - em - 4 - carboxylic acid

Diphenylmethyl 3 - dichloroacetoxymethyl - 7β - (2′ - thienylacetamido)ceph - 3 - em - 4 - carboxylate (6.8 g.) was dissolved in anisole (5 ml.), and trifluoroacetic acid (15 ml.) was added. After 4 minutes the solvent was removed at 30°. The residue was dissolved in ethyl acetate and re-evaporated; the gum was redissolved in ethyl acetate (10 ml.) and added dropwise, with stirring, to petroleum ether (400 ml.). The title compound was obtained as a yellow solid, m.p. > 60° (softens; decomp. at 99°) (4.92 g.; 95%), $[\alpha]_D^{27}=+56°$ (c, 0.7; dioxan), $\lambda\lambda_{max.}$ (ethanol) 237 nm (ϵ=12,500), 259 nm (ϵ=7,500), $\nu_{max.}$ ($CHBr_3$) 3390 (NH), 1788 (β-lactam), 1760 (COOR), 1685 and 1518 (CONH), 1735 and 1715 cm^{-1} (COOH). N.M.R. spectrum ($CDCl_3$) —O.COC*H*Cl_2 4.0 τ.

The dicyclohexylamine salt crystallised from acetone, m.p. > 100° (softens; decomp. at 210°), $[\alpha]_D^{25}=+36°$ (c 1.0, chloroform), $\lambda\lambda_{max.}$ (ethanol) 235 nm (ϵ=13,600), 265 nm (ϵ=6,950), $\nu_{max.}$ ($CHBr_3$) 1774 (β-lactam), 1765 (COOR), 1635 (COO^-), 1680 and 1518 (CONH), 812 cm^{-1} ($CHCl_2$). N.M.R. spectrum ($CDCl_3$)—CH_2.O.COC*H*Cl_2 3.91 τ. (Found C, 52.0; H, 5.7; N, 6.3; Cl, 14.2. $C_{16}H_{14}Cl_2N_2O_6S_2$. $(C_6H_{11})_2NH$ requires C, 52.0; H, 5.8; N, 6.5; Cl, 14.8%).

(c) Diphenylmethyl 7β - amino - 3 - isopropoxymethylceph - 3 - em - 4 - carboxylate, hydrogen p - toluenesulphonate.

3 - Dichloroacetoxymethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 - carboxylic acid (14 g., 30.1 m.mole.) was refluxed in isopropanol (110 ml.) for 45 minutes. After filtration through a pad of kieselguhr, the isopropanol solution was poured into water (1000 ml.) and the pH adjusted to 8.5. The solution was extracted with ethyl acetate (2 × 100 ml.) and the extracts discarded. The solution was then acidified to pH 1.5 with 2N-hydrochloric acid and extracted with ethyl acetate (3 × 100 ml.). The ethyl acetate extracts were dried over magnesium sulphate, and evaporated. The gum was dissolved in ethyl acetate (40 ml.), and ether (60 ml.) added. The precipitate was filtered off and the filtrate evaporated to give an orange gum (5.9 g.).

A solution of the above orange gum (5.9 g.) in tetrahydrofuran (65 ml.) was treated with an excess of diphenyldiazomethane (prepared from 4.5 g. of benzophenone hydrazone) in ether (80 ml.). After one hour the solution was treated with glacial acetic acid (2 ml.), and evaporated. The resulting gum was chromatographed on a silicic acid column (4 × 15 cms.) with (a) benzene and (b) benzene: ethyl acetate=9:1. Those fractions containing a substance R_f *ca.* 0.7 on thin-layer chromatography (silica gel GF 254, with benzene: ethyl acetate=5:1) were combined and evaporated to give a pale yellow gum (1.73 g.), which was diphenylmethyl 3 - isopropoxymethyl - 7β - (2′ - thienylacetamido) - ceph - 3 - em - 4 -

carboxylate. The 7-sidechain was removed and the hydrogen *p*-toluenesulphonate formed by the general method described in Example 1(c), yielding the title compound (600 mg., 3.3% based on the dichloroacetoxymethyl compound) $\lambda_{max.}$ (ethanol) 263 nm (ε 6,600), $\nu_{max.}$ 1792 (β-lactam), 1728 (COOR) and 1130 (SO_3^-) cm.$^{-1}$ (Found: C, 61.1; H, 6.0; N, 4.3; S, 10.3. $C_{31}H_{34}N_2O_7S_2$ requires C, 61.0; H, 5.6; N, 4.6; S, 10.5%), N.M.R. ($CDCl_3$) 5.41 and 5.83 (quartet, J=16 Hz, $—CH_2OCH(CH_3)_2$), 6.62, 8.99 τ ($—CH_2—CH(CH_3)_2$ respectively).

Another experiment afforded *diphenylmethyl 7β - amino - 3 - isopropoxymethyl-ceph - 3 - em - 4 - carboxylate* in a crystalline condition, m.p. 157—161° (d), $[\alpha]_D^{28}$ — 3° (C 1.0; ethanol), after removal of the 7-side-chain, but before the formation of the *p*-toluenesulphonic acid salt.

(d) 7β - (D - α - Amino - α - phenylacetamido) - 3 - isopropoxymethylceph - 3 - em - 4 - carboxylic acid

Acylation of diphenylmethyl 7β - amino - 3 - isopropoxymethyl - ceph - 3 - em - 4 - carboxylate hydrogen - *p* - toluene sulphonate (0.94 g., 1.47 mmole.) with the mixed anhydride from N - (t - butoxycarbonyl) - D - phenylglycine (0.77 g., 3.06 m.mole.) and isobutylchloroformate, by the general method described in Example 1(d), gave the title substance (0.46 g., 74%), $[\alpha]_D^{28}$ + 45° (C 1, H_2O), $\nu_{max.}$ (water) 260 nm (ε 6,900), $\nu_{max.}$ (Nujol) 1766 (β-lactam) and 1695 (COO^-) cm.$^{-1}$, N.M.R. (D_2O) 5.26, 6.02, 8.67 τ ($—CH_2OCH(CH_3)_2$ respectively) R_f 0.76 (system A), 0.18 (system B). This material was contaminated by impurities not revealed on the chromatograms under ultra-violet light; the main impurity is probably α-phenylglycine (*ca.* 30%) (Found: S:N= 1:4.1. Calc. for $C_{19}H_{23}N_3O_5S$: S:N=1:3).

Example 11.

7β - But - 3 - enamido - 3 - methoxymethyl-ceph - 3 - em - 4 - carboxylic acid

A solution of diphenylmethyl 7β - amino - 3 - methoxymethyl - ceph - 3 - em - 4 - carboxylate hydrogen *p* - toluenesulphonate (1.5 g., 2.58 mmole.) in N,N - dimethylacetamide (3 ml.) and acetonitrile (10 ml.) was treated with vinylacetylchloride (1 g., 9.6 mmole.). The solution was kept at room temperature for 15 minutes, when it was evaporated under reduced pressure. The resulting oil was dissolved in ethyl acetate and the solution extracted with aqueous sodium hydrogen carbonate. After evaporation of the solution the crystalline solid was treated with trifluoracetic acid (10 ml.) and anisole (3 ml.). After 8 minutes the reagents were removed under reduced pressure. The residue was dissolved in ethyl acetate (3 ml.) from which the title compound separated as colourless prisms (300 mg., 37%), m.p. 157—161° (decomp.), $[\alpha]_D^{28}$ + 95° (C 1, tetrahydrofuran), $\lambda_{max.}$ (ethanol) 261 nm (ε 6,600), $\nu_{max.}$ (Nujol) 1773 (β-lactam), 1662 and 1540 (—CONH—) and 1700 (COOH) cm.$^{-1}$, N.M.R. (D_2O/$NaHCO_3$) 4.75 (CH_2=$CHCH_2$—), 3.8—4.27 (CH_2=$CHCH_2$—), 6.87 (CH_2=$CHCH_2$—), 5.80 ($—CH_2OCH_3$) and 6.71 τ ($—CH_2OCH_3$). (Found: C, 49.7; H, 5.1; N, 9.1; S, 10.1. $C_{13}H_{16}N_2O_5S$ requires C, 50.0; H, 5.2; N, 9.0; S, 10.3%).

Examples 12—18.

These examples illustrate the acylation of diphenylmethyl 7β - amino - 3 - methoxymethyceph - 3 - em - 4 - carboxylate hydrogen - *p* - toluenesulphonate (prepared as described in Example 1(c)) with a variety of acylating agents. The acylations were carried out in a manner analogous to that described in Example 1(d). The characterising information on the products obtained is given below.

Example 12.

7β - Cyanoacetamido - 3 - methoxymethyl-ceph - 3 - em - 4 - carboxylic acid

m.p. 190—192° (decomp.), $[\alpha]_D^{23}$ + 113° (C 1, tetrahydrofuran), $\lambda_{max.}$ (ethanol) 260—263 nm (ε 7,100), $\nu_{max.}$ (Nujol) 2264 (CN), 1772 (β-lactam), 1720 (—COOH), and 1660 and 1538 cm.$^{-1}$ (CONH), N.M.R. (D_2O, with $NaHCO_3$) 5.78 ($—CH_2OCH_3$), 6.70 ($—O—CH_3$) and 4.32 and 4.80 (protons at the 6- and 7-positions). (Found: C, 46.1; H, 4.2; N, 13.4; S, 10.0. $C_{12}H_{13}N_3O_5S$ requires C, 46.3; H, 4.2; N, 13.5; S, 10.3%), R_f 0.10 (system C).

Example 13.

3 - Methoxymethyl - 7β - trichloroacetamido-ceph - 3 - em - 4 - carboxylic acid (dicyclohexylamine salt)

m.p. 195—200° (decomp.), $[\alpha]_D^{23}$ + 71° (C 1, tetrahydrofuran), $\lambda_{max.}$ (ethanol) 262 nm (ε 6,200), $\nu_{max.}$ (bromoform) 3425 (NH), 1774 (β-lactam), 1722 and 1518 (CONH), and 1632 cm.$^{-1}$ (COO^-), N.M.R. ($CDCl_3$) 5.58 (CH_2OCH_3) and 6.65 τ (CH_2OCH_3). (Found: C, 48.8; H, 5.7; N, 7.2; S, 6.6; Cl, 18.0. $C_{23}H_{34}Cl_3N_3O_5S$ requires C, 48.4; H, 6.0, N, 7.4; S, 5.6; Cl, 18.6%), R_f 0.43 (system C).

Example 14.

3 - Methoxymethyl - 7β - (2',2',2' - trichloroethoxycarbonyl amino) - ceph - 3 - em - 4 - carboxylic acid (dicyclohexylamine salt)

m.p. 171—174° (decomp.), $[\alpha]_D^{23}$ + 69° (C 1, tetrahydrofuran), $\lambda_{max.}$ (ethanol) 263 nm (ε 7,400), $\nu_{max.}$ (bromoform) 1776 (β-lactam), 1522 and 1746 ($NHCO_2.CH_2CCl_3$), 1590 and 1636 (COO^-), and 3445 cm.$^{-1}$ (NH), N.M.R. ($CDCl_3$) 5.17 (CCl_3CH_2O), 5.57 (CH_2—OCH_3), and 6.64 (CH_2OCH_3).

(Found: C, 48.2; H, 5.8; N, 6.9; S, 5.3; Cl, 17.0. $C_{24}H_{36}Cl_3N_3O_6S$ requires C, 48.0; H, 6.0; N, 6.9; S, 5.3; Cl, 17.7%) R_f 0.51 (system C).

Example 15.

7β - S - Benzylthioacetamido - 3 - methoxymethylceph - 3 - em - 4 - carboxylic acid

m.p. 101—106°, $[\alpha]_D^{23}$ + 128° (c 1.0, ethanol), $\lambda_{max.}$ (ethanol) 255—258 nm (ε 7,300), $\nu_{max.}$ ($CHBr_3$) 1790 (β-lactam), 1742 (COOH), and 1684 and 1518 $cm.^{-1}$ (CONH), N.M.R. ($CDCl_3$), 5.59 (—CH_2—O—CH_3), 6.21 (PhCH_2—S—), and 6.64 τ (—O—CH_3).

Example 16.

7β - Bromoacetamido - 3 - methoxymethylceph - 3 - em - 4 - carboxylic acid

m.p. 154° (decomp.), $[\alpha]_D$ + 95° (dioxan, C 1.01), $\lambda_{max.}$ (pH 6.0 phosphate) 258 nm (ε 9,050), $\nu_{max.}$ (Nujol) 1775 (β-lactam), 1725 (—CO_2H), and 1655 and 1550 (CONH), N.M.R. (D_2O, with $NaHCO_3$) 5.82 (—CH_2—O—CH_3), 6.01 (Br—CH_2—CO—NH), 6.71 (—O—CH_3), and 8.82 and 6.35 (0.5 mole. ethanol) (Found: C, 37.5; H, 4.2; Br, 19.9; N, 6.6; S, 8.1. $C_{11}H_{13}BrN_2O_5.0.5\ C_2H_5OH$ requires C, 37.1; H, 4.15; Br, 20.6; N, 7.2; S, 8.3), R_f 0.26 (system B).

Example 17.

7β - (p - Fluorophenoxyacetamido) - 3 - methoxymethylceph - 3 - em - 4 - carboxylic acid

m.p. 146—148°, $[\alpha]_D^{25}$ + 65.9° (c 1.00, dioxan), $\lambda_{max.}$ (pH 6 buffer) 262 nm (ε 9,740), $\nu_{max.}$ (bromoform) 1780 (β-lactam), 1736 (carboxylic acid), and 1692 and 1520 $cm.^{-1}$ (CONH) (Found: C, 52.0; H, 4.3; N, 7.2; S, 8.4; F, 4.6. $C_{17}H_{17}N_2O_6FS_{(396)}$ requires C, 51.5; H, 4.3; N, 7.1; S, 8.1; F, 4.8%).

Example 18.

7β - (2',5' - Dichlorophenylacetamido) - 3 - methoxymethylceph - 3 - em - 4 - carboxylic acid

m.p. 114—125° (dec.), R_p (solvent A), R_p 2.44 (solvent C), $[\alpha]_D^{21}$ + 123° (Me_2SO, C 1.00), $\lambda_{max.}$ (ethanol) 262 nm (ε 6,800) and 225 nm (ε 14,300), $\nu_{max.}$ (Nujol) 3220 $cm.^{-1}$ (NH), 1778 $cm.^{-1}$ (β-lactam) 1710 $cm.^{-1}$ (CO_2H), and 1660 and 1550 cm^{-1} (CONH), τ (in $(D_3C)_2SO$) 2.4—2.7 (3-aromatic protons), 4.28 (1-proton double doublet, J 8.5 and 5 Hz, 7—H), 4.86 (1-proton doublet J 5 Hz, 6H), 5.77; (3-proton singlet, 3—CH_2), 6.24 (2-proton singlet, benzylic methylene), 6.43 (2-proton singlet, 2—CH_2), 6.76 (3-proton singlet, OCH_3) (Found: C, 46.9; H, 3.7; N, 6.4; S, 7.6; Cl, 15.5. $C_{17}H_{16}N_2SO_5Cl_2$ requires C, 47.3; H, 3.7; N, 6.4; S, 7.4; Cl, 16.4%).

Example 19.

2,2,2 - Trichloroethyl 7β - amino - 3 - methylceph - 3 - em - 4 - carboxylate, hydrogen p - toluenesulphonate

The 7-side chain was removed in a manner similar to that described in Example 1(c) from 2,2,2-trichloroethyl 3 - methyl - 7β - phenylacetamidoceph - 3 - em - 4 - carboxylate (1.0 g., 2.16 mmole.), $\lambda_{max.}$ (ethanol) 263 nm. (ε 6,000), N.M.R. ($CDCl_3$) 7.82 τ (3—CH_3), to give the title compound (0.27 g., 24%), $\lambda_{max.}$ (ethanol) 262 nm (ε 6,200). $\nu_{max.}$ (Nujol) 1766 (β-lactam), 1720 cm^{-1} (—CO_2R), N.M.R. ($CDCl_3$) 7.67 τ (3—CH_3) in an impure state.

Example 20.

Preparation of 3 - Acetoxymethyl - 7β - aminoceph - 3 - em - 4 - carboxylic acid

The title compound was prepared via the following reaction scheme (the steps are described in more detail below)

3 - Acetoxymethyl - 7β - (5' - carboxy - 5' - oxopentanamido) - ceph - 3 - em - 4 - carboxylic acid

↓ (a)

3 - Acetoxymethyl - 7β - (5' - carboxy - 5' - hydroxypentanamido) - ceph - 3 - em - 4 - carboxylic acid

↓ (b)

Diphenylmethyl 3 - acetoxymethyl - 7β - (5' - diphenylmethoxycarboxyl - 5' - hydroxypentanamido) - ceph - 3 - em - 4 - carboxylate

↓ (c)

3 - Acetoxymethyl - 7β - aminoceph - 3 - em - 4 - carboxylic acid.

(a) 3 - Acetoxymethyl - 7β - (5' - carboxy-5' - hydroxypentanamido) - ceph - 3-em-4-carboxylic acid

3 - Acetoxymethyl - 7β - (5' - carboxy-5' - oxopentanamido) - ceph - 3 - em - 4 - carboxylic acid [4.12 g., 0.01 mole.; $\lambda_{max.}$ (pH 6 buffer) 260 nm. (ε 5,270), $R_{ceph\ c}$ 1.14, trace spots at $R_{ceph\ c}$ 0.45, 3.73 (system C)] was suspended in water (100 ml.) and the pH was adjusted to 8.0 by the addition of 0.1*N*-sodium hydroxide. The resulting solution was kept at 0° while sodium borohydride (0.38 g., 0.01 mole.) was added; glacial acetic acid was also added to keep the pH at 8.0. The solution was stirred at 0° for 10 min. then Dowex (Registered Trade Mark) 50 resin (H^+ form) was added until the pH was 2.0. The mixture was stirred for a further 10 min., filtered to remove the resin and the filtrate freeze-dried giving a fluffy solid which was stirred in ether (3 × 400 ml.) then collected by filtration giving the title compound as a colourless powder (2.85 g) $R_{ceph\ c}$ 2.18, $\lambda_{max.}$ (pH 6 buffer; 29337) 258 nm. (ε 6,000), $\nu_{max.}$ (Nujol) 1780 (β-lactam), 1738 (acetate), 1670, 1540 $cm.^{-1}$ (amide), τ (D_2O with $NaHCO_3$)

4.33 (1-proton doublet J 5 c./sec.; C—7H), 4.85 (1-proton doublet, J 4.5 c./sec.; C—6H), 7.87 (3-proton singlet; CH_3CO).

(b) Diphenylmethyl 3 - acetoxymethyl - 7β - (5′ - diphenylmethoxycarbonyl - 5′ - hydroxypentanamido) - ceph - 3 - em - 4 - carboxylate

Benzophenone hydrazone (9.82 g., 0.05 mole) and mercuric oxide (10.84 g., 0.05 mole) in petroleum-ether (175 ml., b.p. 40—60°) were stirred in the dark for 6 hrs. at room temperature. The mixture was filtered and the filtrate made up to 200 ml. with petroleum-ether. A portion (30 ml.) of this solution was added to a suspension of 3 - acetoxymethyl - 7β - (5′ - carboxyl - 5′ - hydroxypentanamido) - ceph - 3 - em - 4 - carboxylic acid (1,04 g., 0.0025 mole) in dioxan (50 ml.), and the mixture stirred at room temperature overnight. The solvents were evaporated from the resulting solution, leaving a purple oil which was dissolved in ethyl acetate (100 ml.). This solution was washed with 2N-hydrochloric acid (100 ml.), 3%-sodium hydrogen carbonate (100 ml.) and water (2 × 100 ml.), dried (magnesium sulphate), and the solvent evaporated off, leaving a yellow gum (1.41 g.). This was stirred in ether (300 ml.) for 7 hrs.; the small amount of solid remaining undissolved was removed by filtration, and the filtrate was evaporated leaving the title compound as a yellow foam (1.07 g.), TLC R_f 0.88 (silica-gel plates, acetone as developing solvent), $\lambda_{max.}$ (ethanol) 260 nm. (ε 7,000), $\nu_{max.}$ (bromoform) 1780 (β-lactam), 1730 cm^{-1} (esters).

(c) 3 - Acetoxymethyl - 7β - aminoceph - 3 - em - 4 - carboxylic acid

Diphenyl methyl 3-acetoxymethyl-7β-(5′-diphenylmethoxycarbonyl - 5′ - hydroxypentanamido)-ceph - 3 - em - 4 - carboxylate (1.07 g., 0.0043 mole) was treated with phosphorus pentachloride (0.90 g., 0.0043 mole) and pyridine (1 ml.) in methylene dichloride (24 ml.) as in Example 6(b). Continuation of the procedure described previously resulted in the isolation of the title compound (0.02 g., 5%), with paper chromatographic behaviour and infra-red spectrum identical with authentic material.

Example 21.

3 - Acetoxymethyl - 7β - aminoceph - 3 - em - 4 - carboxylic acid

A suspension of 3 - acetoxymethyl 7β - (4′ - carboxybutanamide) - ceph - 3 - em - 4 - carboxylic acid (772 mg., 0.002 mole) in methylenedichloride (7 ml) was solubilised by the addition of triethylamine (0.55 ml., 0.0039 mole), and trimethylchlorosilane (0.515 ml., 0.0045 mole) then added to the resulting solution. The reaction mixture was stirred for 2 hrs., pyridine (1.92 ml., 0.024 mole) added and, after cooling to −10°, a solution of phosphorus pentachloride (1,246 g., 0.006 mole) in methylenedichloride (12 ml) added dropwise. The suspension was stirred at −10° for 35 mins., ice-cold methanol (5 ml) added dropwise, and the solution stirred at 0° for a further 3 hrs. before being poured into water (2 ml). The pH was adjusted to 3.5 with 6N-sodium hydroxide and, after the two-phase system had stood at 0° for 16 hrs. the precipitated title compound (0.033 g. 6— $[\alpha]_D^{20°}$ + 85.9° (C 0.99, 0.2N, pH 7.0-phosphate buffer) $\lambda_{max.}$ 261 nm (ε 6,900), pH 6 phosphate buffer, was isolated by filtration. Electrophoresis at pH 1.9 showed the sample to be identical with authentic material.

Example 22.

3 - Acetoxymethyl - 7β - aminoceph - 3 - em - 4 - carboxylic acid

Trimethylchlorosilane (0.515 ml., 0.0045 mole) was added to a solution of 3 - acetoxymethyl 7β - (4′ - carboxybutanamido) - ceph - 3 - em - 4 - carboxylic acid (777 mg., 0.002 mole) in chloroform (7 ml) containing triethylamine (0.55 ml., 0.0039 mole) and, after stirring at room temperature for 2 hrs., pyridine (0.64 ml., 0.008 mole) added, the solution cooled to −10° and treated with a solution of phosphorus pentachloride (456 mg., 0.0022 mole) in chloroform (10 ml) over 2 mins. The reaction mixture was stirred at 0° for 30 mins., ice-cold methanol (5 ml) added dropwise at −10°, and the solution stirred at 0° for a further 3 hrs. before being poured into water (2 ml). The pH was adjusted to 3.5 with 6N-sodium hydroxide and after the two-phase system had stood at 0° for 16 hrs., the precipitated title compound (0.105 g., 20%) $[\alpha]_D^{20°}$ + 92.7° (C 1.11, 0.2M, pH 7.0-phosphate buffer) $\lambda_{max.}$ 262 nm (ε 6,800) ph 6 phosphate buffer was isolated by filtration. Electrophoresis at pH 1.9 showed the sample to be identical with authentic material.

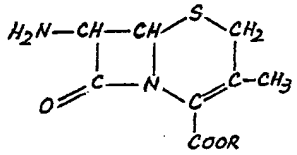
Example 23.

3 - Acetoxymethyl - 7β - aminoceph - 3 - em - 4 - carboxylic acid

Trimethylchlorosilane (0.175 ml., 0.0015 mole) was added to a solution of 3 - acetoxymethyl 7β - pentanamidoceph - 3 - em - 4 - carboxylic acid (0.363 g., 0.001 mole) in methylenedichloride (5 ml) containing triethylamine (0.14 ml. 0.001 mole) and, after stirring at room temperature for 2½ hrs., pyridine (0.32 ml., 0.04 mole) added, the solution cooled to −10° and treated with a solution of phosphorus pentachloride (0.254 g., 0.0012 mole) in methylenedichloride (5 ml), over 5 mins. The reaction mixture was stirred at 0° for 30 mins., ice-cold methanol (5 ml) added dropwise at −10°, and the solution stirred at 0° for a further 2½ hrs. before being poured into water (2 ml). The two-phase system was stirred at room temperature for 10

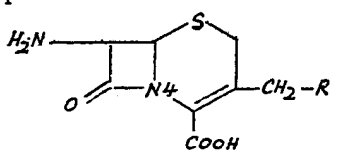
mins., the pH adjusted to 3.6 with 6N-sodium hydroxide and, after standing at 0° for 16 hrs., the precipitated title compound (0.195 g., 72%) was isolated by filtration, $[\alpha]_D^{200}$ + 87.6° (C 0.97, 0.2M, pH 7.0-phosphate buffer), $\lambda_{max.}$ 262 nm (ε 7,600). Electrophoresis at pH 1.9 showed the sample to be identical with authentic material.

In Specification No. 1,227,014 there is claimed a process for preparing 7 - amino - desacetoxycephalosporanate ester compounds of the formula:



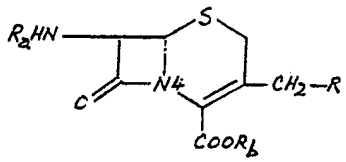
wherein R is 2,2,2-trichloroethyl, benzyloxymethyl, tert-butyl, p-methoxyphenyl, 3,5 - dimethoxybenzyl, benzhydryl, bis(3,5 - dimethoxyphenyl)methyl or p - methoxybenzyl; and toluenesulfonic and naphthalenesulfonic acid salts of said esters useful as intermediates in the preparation of 7 - acylamidodesacetoxycephalosporanic acid antibiotics, which comprises (a) reacting phosphorus pentachloride, phosphorus oxychloride or an equivalent halogenating agent with the 2,2,2 - trichloroethyl, benzyloxymethyl, tert - butyl, p - methoxyphenyl, 3,5 - dimethoxybenzyl, benzhydryl, bis(3,5 - dimethoxyphenyl)methyl or p - methoxybenzyl ester of 7 - acylamidodesacetoxycephalosporanic acid in a non-hydroxylated anhydrous organic liquid solvent at a temperature of from about 40°C to about 80°C in the presence of about 1 equivalent of basic neutralizing agent for from 1 to 1.5 moles of phosphorus halide to form an imino-halide of the 7-acylamidodesacetoxycephalosporanate, (b) commingling or mixing an alcohol with the imino-halide to form an imino-ether hydrochloride of the 7-acylamidodesacetoxycephalosporanate ester, and (c) commingling water with the imino-ether hydrochloride to form the 7-aminodesacetoxycephalosporanate ester and if desired adding to the 7-aminodesacetoxycephalosporanate ester product an aromatic hydrocarbon sulfonic acid having from 6 to about 12 carbon atoms in the aromatic hydrocarbon moiety to form an aromatic hydrocarbon sulfonate salt thereof.

In Specification No. 1,239,814 there is claimed a process for the preparation of a compound of the formula



(I)

wherein R is hydrogen or an esterified hydroxyl group, which comprises treating a 4-silyl ester of the formula



(II)

wherein R is as specified above, R_a is an acyl group having any functional groups present blocked, and R_b is a silyl group, with an agent to form a 7-imino halide at a temperature below −15°C, treating the 7-imino halide with an agent to form a 7-imino ether at a temperature below −15°C, and hydrolysing or alcoholysing the product obtained to split the imino ether and to liberate the compound of formula (I).

No claim is made herein to any process (i) in which the imide halide formation is carried out at a temperature of from about 40° to about 80°C in the case where the carboxyl group at the 4 - postion of a 7β - acylamino - 3 - methyl - 4 - carboxy cephalosporin compound used as starting material is blocked with a 2,2,2-trichloroethyl, benzyloxy-methyl, tert-butyl, p-methoxyphenyl, 3,5-dimethoxybenzyl, benzhydryl, bis(3,5-dimethoxyphenyl)-methyl or p-methoxybenzyl ester group and (ii) in which the imide halide formation and the imino ether formation is carried out at a temperature below −15°C in the case where the carboxyl group at the 4-position of a 7β-acylamino-3-methyl-, or 3-esterified hydroxymethyl-4-carboxy cephalosporin compound used as starting material is blocked with a silyl group.

Subject to the foregoing disclaimer,

WHAT WE CLAIM IS:—

1. A process for the N-deacylation of a 7β-acylamino-4-carboxy cephalosporin compound (other than one having at the 7-position a δ-aminoadipoylamino group or a δ-aminoadipoylamino group having one or other or both of the amino and carboxy groups thereof blocked) which comprises converting said compound into an imide halide by means of an imide halide forming reagent, converting the imide halide so formed into an imino ether and hydrolysing or alcoholysing the latter, to produce a resulting 7β-amino cephalosporin compound.

2. A process as claimed in claim 1 wherein said imide halide forming reagent is an acid halide derived from a phosphorus acid.

3. A process as claimed in claim 2 wherein said imide halide forming reagent is phosphorus oxychloride or phosphorus pentachloride.

4. A process as claimed in any of the preceding claims carried out in the presence of an inorganic or organic base.

5. A process as claimed in claim 4 wherein said organic base is a tertiary amine.

6. A process as claimed in claim 5 wherein said tertiary amine is triethylamine, pyridine or dimethylaniline.

7. A process as claimed in claim 4 wherein said inorganic base is calcium carbonate.

8. A process as claimed in any of the preceding claims wherein the imide halide formation is effected in solution in a chlorinated hydrocarbon solvent.

9. A process as claimed in claim 8 wherein said chlorinated hydrocarbon is methylene chloride.

10. A process as claimed in any of the preceding claims in which the imide halide forming reagent is introduced into the reaction mixture in the form of a solution in an organic solvent.

11. A process as claimed in any of the preceding claims wherein said imide halide is converted into the imino ether by reaction with an alcohol in the presence of a tertiary amine.

12. A process as claimed in claim 11 wherein said alcohol is methanol.

13. A process as claimed in any of the preceding claims wherein said imino ether is hydrolysed or alcoholysed by using water or an alkanol in the presence of a basic or acidic catalyst.

14. A process as claimed in claim 13 wherein said acidic catalyst is a mineral or organic acid.

15. A process as claimed in claim 14 wherein said mineral or organic acid is hydrochloric acid, trifluoroacetic acid, *p*-toluenesulphonic acid, phosphoric acid or formic acid.

16. A process as claimed in claim 13 wherein said basic catalyst is ammonia or the salt of a weak acid with an alkali metal or alkaline earth metal.

17. A process as claimed in any of the preceding claims wherein the 4-position carboxyl group of said 7β - acylamino - 4 - carboxy - cephalosporin compound is blocked by a diphenylmethyl, *t*-butyl or β,β,β - trichloroethyl esterifying group.

18. A process as claimed in claim 17 wherein said diphenylmethyl or said *t*-butyl esterifying group is subsequently removed by means of a mixture of trifluoroacetic acid and anisole at room temperature.

19. A process as claimed in claim 17 wherein said β,β,β-trichloroethyl esterifying group is subsequently removed by Zn/acetic acid.

20. A process as claimed in claim 17 wherein said diphenylmethyl esterifying group is introduced by means of diphenyldiazomethane.

21. A process as claimed in any of the preceding claims wherein the resultant 7β-aminocephalosporin compound is recovered in the form of an acid addition salt thereof.

22. A process as claimed in claim 21 wherein said acid addition salt is the hydronitrate or hydrogen hydrocarbyl sulphonate.

23. A process as claimed in claim 22 wherein said hydrogen hydrocarbyl sulphonate is an alkylbenzene sulphonate or a lower (C_1—C_6) alkane sulphonate.

24. A process as claimed in claim 23 wherein said hydrogen hydrocarbyl sulphonate is the hydrogen *p*-toluene sulphonate or the hydrogen methane sulphonate.

25. A process as claimed in any of the preceding claims wherein the acyl group of said 7β - acylamino - 4 - carboxy cephalosporin compound is an acyl group of the class (i) herein defined.

26. A process as claimed in any of claims 1—24 wherein the acyl group of said 7β-acylamino-4-carboxy cephalosporin compound is an acyl group of the class (ii) herein defined.

27. A process as claimed in any of claims 1—24 wherein the acyl group of said 7β-acylamino-4-carboxy cephalosporin compound is an acyl group of the class (iv) herein defined.

28. A process as defined in any of claims 1—24 wherein said 7β - acylamino - 4 - carboxy - cephalosporin compound is a 7β-phenylacetamido compound.

29. A process as defined in any of claims 1—24 wherein said 7β - acylamino - 4 - carboxy - cephalosporin compound is a 7β - phenoxyacetamido compound.

30. A process as defined in any of claims 1—24 wherein said 7β - acylamino - 4 - carboxy - cephalosporin compound is a 7β - thienylacetamido compound.

31. A process as claimed in any of the preceding claims in which said 7β - acylamino - 4 - carboxy - cephalosporin compound possesses an etherified hydroxymethyl group at the 3-position.

32. A process as claimed in claim 31 in which said etherified hydroxymethyl group is a methoxymethyl group.

33. A process as claimed in any of the preceding claims including the additional step of re-acylating said 7β-amino cephalosporin compound to produce a 7β-acylamino cephalosporin compound other than the 7β-acylamino compound used as starting material in the N-deacylating process.

34. A process as claimed in claim 1 substantially as herein described with reference to the Examples.

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